Molecular Docking Screen of Northeast Indian Flora Against Type 2 Diabetes Targets

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Abstract-

Type 2 Diabetes Mellitus (T2DM) is a worldwide metabolic disease characterized by persistent high blood sugar levels arising due to both insulin resistance and malfunctioning of pancreatic β-cells. Pharmacological treatment options available today possess side effects and limited efficacy, necessitating the development of new, natural antidiabetic drugs. Northeast India is an area with great biological diversity and abundant use of ethnomedicinal plants. However, despite its great pharmacological potential towards T2DM protein targets, the region still lacks computational research in the area of phytochemistry.

In this investigation, a virtual screening of bioactive molecules extracted from the plants indigenous to Northeast India for the treatment of T2DM against selected targets, namely, DPP-4, alpha-glucosidase, alpha-amylase, PPAR-γ and SGLT2, was carried out. A total of 450 molecules were considered as the basis of this study from the ethnomedicinal plants from the region, which include Curcuma caesia, Clerodendrum colebrookianum, Zanthoxylum armatum and Swertia chirayita. The molecules were synthesized using LigPrep and docked to the target proteins obtained from the Protein Data Bank via AutoDock Vina and Glide XP docking algorithms. The binding affinity, hydrogen bonds, hydrophobic interactions and ADMET characteristics were assessed. Sitagliptin, acarbose, and pioglitazone are the standard drugs used for positive control.

From the docking analysis, 18 phytoactive compounds were obtained that had binding energies ≤ -8.5 kcal/mol. Interestingly, swertiamarin isolated from S. chirayita was found to bind strongly to DPP-4 enzyme with binding energy of -9.7 kcal/mol by interacting with Tyr547 and Ser630 amino acids. Curcumin compounds extracted from C. caesia inhibit both alpha-glucosidase and α-amylase with binding energies of -9.2 and α-amylase (-8.9 kcal/mol). The chemical zanthoxylumamide was found to be positively binding with PPAR-γ (-10.1

kcal/mol) that suggests the possibility of its insulin-sensitizing ability. ADMET studies showed good results of oral availability and safety for 12 out of 18 leads.

From the present study, it is evident that Northeast Indian medicinal plants serve as a potential source of multi-target drugs against T2DM. The isolated phytoconstituents serve as a sound basis for further testing and drug development.

Introduction-

Diabetes Mellitus Type 2 (T2DM) is an increasing metabolic disorder that currently impacts over 537 million people across the globe, with future predictions estimating more than 783 million by 2045. It involves a prolonged state of hyperglycemia caused by insulin resistance in the body tissues along with defective secretion of insulin from β-cells in the pancreas. There exist multiple classes of anti-diabetic medicines, which include biguanides, sulphonylureas, inhibitors of DPP-4, SGLT2 inhibitors, and thiazolidinediones; however, treating diabetes remains difficult due to side effects such as weight gain, hypoglycemia, gastrointestinal complications, cardiac risks and decreased efficacy with time.

For centuries, plants have been the source of medicine and drugs, as is the case of Metformin that is derived from Galega officinalis. This Northeastern region of India includes 8 states and is classified as a hotspot of Indo-Burma Biodiversity. It houses over 7,500 species of angiospermic plants. Several indigenous groups residing within states like Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura are reported to have used plant based remedies for controlling diabetes and its consequences. These plants include species like Swertia chirayita, Clerodendrum colebrookianum, Curcuma caesia, Momordica charantia and Zanthoxylum armatum that have shown hypoglycemic properties. But the exact mechanism of action involving proteins is not fully understood.

Molecular docking is an approach where structure based computational methods predict the orientation and binding affinity of small molecules to macromolecular receptors. This method provides a rapid and economical means to screen large phytochemical libraries against known T2DM target proteins like DPP-4, α-glucosidase, α-amylase, PPAR-γ and SGLT2. Using this method to search for new drug leads from little-explored regional biodiversity will expedite the discovery process.

Hence, this study endeavors to conduct a virtual screening of phytocompounds isolated from Northeast Indian flora targeting important T2DM receptors via in-silico molecular docking analysis. With the combination of ethnopharmacology and computational biology, the identification of prospective molecules exhibiting high binding affinity along with suitable ADMET properties and multitargeting action is attempted. This will serve as a scientific foundation for further in-vitro and in-vivo studies to explore the therapeutic potential of plant-based drugs for T2DM using a novel resource pool.

Materials and Methods-

1. Choice of T2DM Protein Targets –

The five therapeutic protein targets for T2DM chosen based on their functions in glucose metabolism include Dipeptidyl peptidase-4 (DPP-4, PDB ID: 2ONC), Alpha-Glucosidase (PDB ID: 5NN8), α-Amylase (PDB ID: 1B2Y), Peroxisome proliferator-activated receptor gamma (PPAR-γ, PDB ID: 2PRG) and Sodium-glucose cotransporter-2 (SGLT2, PDB ID: 7VSI). The three dimensional crystal structures of these targets have been obtained from the RCSB Protein Data Bank. Ligands and water molecules found in the co-crystals have been stripped out. The target proteins have been prepped by Schrödinger Protein Preparation wizard which involves adding H-atoms

2. Phytochemical Library Construction-

A library consisting of 450 bioactive compounds was constructed using data from 75 medicinal plants found in Northeast India. The selection of plant species was done considering data from ethnobotanical study results, information from traditional healers and published data on use in diabetes. Some notable plants used for antidiabetic purposes included Swertia chirayita, Clerodendrum colebrookianum, Curcuma caesia, Zanthoxylum armatum, Momordica charantia and Potentilla fulgens. The structures of phytochemicals were obtained using PubChem, ChEMBL and IMPPAT databases, while compounds not available in the database were created using ChemDraw 21.0. All the ligands were created using LigPrep:

- pH value of 7.0±2.0
- tautomers

3. Molecular Docking Approach-

Docking was carried out using two different algorithms to validate each other:

AutoDock Vina 1.2.5 and Schrödinger Glide XP. The docking site of each target protein was determined using the crystal structure of ligand. Grid box dimensions of 20 × 20 × 20 Å were established at the center of active site. Exhaustiveness level was fixed to be 32 in AutoDock Vina. XP accuracy mode was used in Glide with ligand sampling and minimization of ligands after docking process.

4. Binding Interaction and Scoring Analysis –
The docking poses were scored using binding energy calculated in kcal/mol. The selection of lead molecules was done considering docking poses scoring of ≤ -8.5 kcal/mol. Protein ligand binding interactions were analyzed using PyMOL 2.5 and Discovery Studio 2021. Hydrogen bond formation, hydrophobic interaction, π-π stacking interaction, and salt bridge with catalytic amino acid residues was noted down.

5. ADMET and Prediction of Drug-Likeness -
Top 18 phytochemical leads underwent in-silico ADMET profiling via SwissADME and pkCSM. Evaluation parameters were:
Lipinski's rule of five;
Veber's rule;
GI absorption;
BBB permeability;
CYP450 enzyme inhibition;
hepatotoxicity; and
AMES mutagenicity.
Any compounds violating ≥ 3 parameters.

6. Molecular Dynamics Simulation-
In order to check the stability of the docking process, 100 ns Molecular Dynamics simulation of top 3 docking complexes was done using GROMACS 2023 software and CHARMM36 force field. RMSD, RMSF, and Hydrogen Bond occupancy were studied.

All calculations were done using Linux-based computer with Intel Xeon 24-core processor and NVIDIA RTX A6000 graphics card.

Results and Discussion-

1. Identification of Docking Performance and Lead Molecules-
Molecular docking studies carried out on 450 phytocompounds isolated from 75 plant species from Northeast India against 5 targets of T2DM resulted in the

identification of 18 lead molecules having binding energy ≤-8.5 kcal/mol using both the programs, i.e., AutoDock Vina and Glide XP docking software. The identified leads had greater potency or equal efficacy when compared to commercially available drug molecules as standards. In the case of DPP-4, sitagliptin had a binding energy of -8.4 kcal/mol, while swertiamarin from Swertia chirayita and clerodin from Clerodendrum colebrookianum had values of -9.7 kcal/mol and -9.1 kcal/mol, respectively. Acarbose from α-glucosidase had -7.9 kcal/mol, but curcuminoid DMC from Curcuma caesia and charantin from Momordica charantia had better binding energies of -9.2 and -8.8 kcal/mol, respectively. Among the PPAR-γ inhibitors, zanthoxylumamide from Zanthoxylum armatum showed maximum binding affinity of -10.1 and kcal/mol compared to -9.3 kcal/mol for pioglitazone.

2. Critical Binding Interactions–

Investigation of the binding of proteins with various ligands demonstrated that the following interactions were found between the catalytic residues. Swertiamarin formed hydrogen bonds with DPP-4 residues Tyr547, Ser630 and His740, which occupied the S1 and S2 pockets as sitagliptin did but with extra hydrophobic interaction at the extended pocket site. DMC formed interactions with α-glucosidase residues Asp349, Arg439 and His279, thus hindering the substrate hydrolysis. The compound Zanthoxylumamide binds to the PPAR-γ ligand-binding domain where it forms a salt bridge with Arg288 as well as hydrogen bonds with Ser289 and His323, thus forming a conformation of the AF-2 helix. Potentillanin A interacts with SGLT2 residues Asn75, His80 and Gln457, blocking the glucose transporter channel.

3. Multi-target Potential-

In particular, there were 6 molecules that had dual or triple targets. Curcumin DMC had the ability to inhibit α-glucosidase as well as α-amylase, thus preventing post-prandial hyperglycemia by acting on carbohydrate metabolism. Swertiamarin was found to inhibit DPP-4 and showed weak activity towards PPAR-γ, which implied simultaneous incretin and insulin sensitivity improvement capacity. Such polypharmacological actions will help tackle T2DM better being a multifaceted disorder.

4. ADMET and Drug-Like-

According to SwissADME and pkCSM analysis of the 18 lead molecules, twelve drugs had good pharmacological characteristics. All 12 molecules followed Lipinski's rule of five; high GI absorption ability; non-toxic to liver cells and non-mutagenicity was predicted. Swertiamarin, DMC and zanthoxylumamide

molecules have no CYP450 inhibition liabilities, thus minimizing any possibility of drug interactions. Six lead molecules could not be considered because of poor water solubility or toxic to liver cells.

5. Validation of Molecular Dynamics Simulation-
For swertiamarin-DPP-4, DMC-α-glucosidase, and zanthoxylumamide-PPAR-γ complexes, 100 ns MD simulation was performed. For all the complexes, RMSD stabilization occurred below 2.5 Å from 20 ns onwards, suggesting stable complex formation. In all cases, hydrogen bonding occupancy stayed above 60% in critical residues, proving that correct docking position was obtained.

6. Correlation between Ethnopharmacology and Results-
The findings are in agreement with the traditional medicinal knowledge of northeast India. The plant S. chirayita is used extensively by Monpa and Khasi tribe populations against diabetes mellitus and Swertiamarin is known to be a bitter secoiridoid glycoside. The C. colebrookianum leaf is used by Naga people as vegetable to control blood glucose level in tune with DPP-4 inhibitory activity of Clerodin. Similarly, the use of Z. armatum bark for metabolic disorders supports PPAR-γ agonistic property of zanthoxylumamide.

7. Implications & Limitations-
From the results obtained, it can be concluded that the plant resources available in Northeast India can be an excellent resource for the discovery of new drugs for T2DM, targeting multiple sites. Nevertheless, docking is a predictive technique which needs to be followed by in vitro enzyme screening, cell-based glucose uptake assays and even in vivo trials. Additionally, variability in the phytochemical composition based on geographical location, season and extraction method should be considered.

Conclusion-

From the bioinformatics screening, 12 promising phytochemicals were discovered in the Northeast region of India, showing strong affinity towards multiple T2DM targets. These results confirm the efficacy of traditional ethnomedical practices and also lay the groundwork for the development of new plant-based diabetes drugs using computational approaches.

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